APPEAL BRIEF
Patent Application
Docket No. BB.124

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Mohammad Y. Meah, Ph.D.
Art Unit	:	1652
Applicants	:	Martin Krause, Christian Scheler, Ulrike Bottger, Hardy Weisshoff, Michael Linscheid
Serial No.	:	10/518,727
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Conf. No.	:	1308
For	:	Method and Reagent for Specifically Identifying and Quantifying One or More Proteins in a Sample

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APPEAL BRIEF

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I. REAL PARTY IN INTEREST

This application is owned by Proteome Factory AG and Humboldt-Universitat Zu Berlin, as evidenced by the assignment set forth at Reel 061539, Frame 0807.

II. RELATED APPEALS AND INTERFERENCES

Appellants do not know of any prior or pending appeals, judicial proceedings, or interferences which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

Claims 1-6, 8, 22 and 24-40 are rejected. Claims 7, 9-21 and 23 have been canceled. The rejection of claims 1-6, 8, 22 and 24-40 is appealed herein.

IV. STATUS OF AMENDMENTS

An after-final amendment was submitted on June 4, 2010. By that amendment, claims 1 and 6 were amended. The advisory action of July 8, 2010 indicates that the proposed amendment will be entered, and that claims 1-6, 8, 22 and 24-40 remain rejected.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The present invention relates to a method and a reagent suitable for performing the method, wherein the method pertains to a reproducible, systematic, qualitative and quantitative proteome characterization by means of non-isotope metal coded markers and - among other items - the most modern tandem methods of mass spectrometry (specification, page 1, paragraph 1).

Thus, the appealed claims are directed to a method for the identification and/or quantification of one or more proteins derived from the proteome of a cell in a sample containing a mixture of such proteins, wherein said method comprises the steps of:

a) providing a sample which contains a mixture of proteins (see, for example, original claim 1 and specification at paragraph bridging pages 5-6);

b) providing a reagent for the analysis of peptides which comprises A, Y and PRG in which

A constitutes at least one functional group for the reversible, covalent or non-covalent binding to a support material,

Y is a group comprising at least one chelate function for metals, and comprising a metal ion bound thereto wherein the metal is selected from the group consisting of Ag, Al, As, Au, Be, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Hg, Ho, In, La, Li, Lu, Mn, Na, Nd, Ni, Pb, Pr, Rb, Rd, Sb, Sm, Sn, Tb, Tl, Tm, V, W, Y, Yb and Zn;

PRG is a reactive group for the selective binding to peptides or other biomolecules to be analyzed; and wherein the arrangement of A, Y, and PRG is interchangeable and said reagent is not isotopically labeled (Abstract, original claim 1, claim 10, claims 9-18 and specification at paragraph bridging pages 5-6 and page 6, paragraph 2);

c) chemically or proteolytically cleaving the proteins in the sample in order to produce peptides (original claim 1 and specification at page 7 paragraph 1);

d) coupling the peptides to the reagent of step b) wherein the peptides are labeled by the reagent (original claim 1 and specification at page 6, paragraphs 2-4);

e) selecting the peptides labeled in step d) using a functional group for the reversible, covalent or non-covalent binding to a support material and removal of unbound peptides (original claim 1, paragraph bridging pages 5-6);

f) releasing the bound peptides from the support material and elution from the matrix (original claim 1 and specification at paragraph bridging pages 5-6); and

g) detecting and identifying the labeled peptides by means of mass spectrometry (original claim 1 and specification at paragraph bridging pages 5-6).

A second independent claim (claim 6) relates to a method for the detection of the relative expression of proteins in a protein-containing sample, wherein said method comprises the steps of:

a) providing a biological sample which contains proteins (original claim 6 and specification in the paragraph bridging pages 7-8);

b) providing a reagent for the analysis of peptides which comprises A, Y and PRG in which

A constitutes at least one functional group for the reversible, covalent or non-covalent binding to a support material,

Y is a group comprising at least one chelate function for metals, and comprising a metal ion bound thereto wherein the metal is selected from the group consisting of Ag, Al, As, Au, Be, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Hg, Ho, In, La, Li, Lu, Mn, Na, Nd, Ni, Pb, Pr, Rb, Rd, Sb, Sm, Sn, Tb, Tl, Tm, V, W, Y, Yb and Zn,

PRG is a reactive group for the selective binding to peptides or other biomolecules to be analyzed and said reagent contains no isotopes; and wherein the arrangement of A, Y, and PRG is interchangeable and said reagent is not isotopically labeled (original claims 6 and 8-19, specification in the paragraph bridging pages 7-8, page 8, paragraph 3 through page 10, paragraph 2);

c) chemically or proteolytically cleaving the proteins in the sample in order to produce peptides (original claim 6 and specification at page 7, paragraph 1);

d) coupling the peptides to the reagent of step b) wherein the peptides are labeled by the reagent (original claim 6 and specification at page 6, paragraphs 2-4);

e) selecting the peptides labeled in step d) utilizing a functional group for the reversible, covalent or non-covalent binding to a support material and removal of the unbound peptides (original claim 6 and paragraph bridging pages 7-8);

f) releasing the bound peptides from the support material and elution from the matrix (original claim 6 and paragraph bridging pages 7-8);

g) detecting and identifying the labeled peptides by means of mass spectrometry (original claim 6 and paragraph bridging pages 7-8); and

h) measuring the relative occurrence of the differently labeled peptides as distinct peaks of ions in order to determine the relative expression of the protein, from which the labeled peptide is derived (original claim 6 and paragraph bridging pages 7-8).

The following table sets forth the portions of the as-filed specification providing support for each of the pending claims:

Claim	Support
2	Original claim 2 and page 7, paragraph 1
3	Original claim 3, page 7, paragraph 2
4	Original claim 4, page 7, paragraph 3
5	Original claim 5 and page 7, paragraph 4
8	Original claim 8, and page 8, paragraph 2
22	Page 6, paragraph 3, Figure 2 and the description thereof
24	Page 9, paragraphs 1-2
25	Page 9, paragraphs 1-2
26	Page 9, paragraphs 1-2
27	Page 9, paragraph 4
28	Page 9, paragraph 3 and paragraph bridging pages 9-10
29	Page 6, paragraph 3, Example, Figure 2 and the description thereof at page 18
30	Page 9, paragraphs 1-2
31	Page 9, paragraphs 1-2
32	Page 9, paragraphs 1-2
33	Page 9, paragraph 4
34	Page 9, paragraph 3 and paragraph bridging pages 9-10
35	Page 10, paragraph 1
36	Page 10, paragraph 1
37	Page 10, paragraph 1
38	Page 10, paragraph 1
39	Page 10, paragraph 2
40	Page 10, paragraph 2

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A) Whether claims 1-6, 8, 22 and 24-40 are unpatentable under 35 U.S.C. § 103(a) as obvious over Aebersold *et al.* (WO 00/11208) in view of Moutiez *et al.* (1997) and Li *et al.* (1997).

VII. ARGUMENT

A. Claims 1-6, 8, 22 and 24-38 are patentable because the claimed invention is not obvious over Aebersold *et al.* (WO 00/11208) in view of Moutiez *et al.* (1997) and Li *et al.* (1997).

Claims 1-6, 8, 22 and 24-38 stand rejected and new claims 39-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Aebersold *et al.* (WO 00/11208, from IDS) in view of Moutiez *et al.* (Analyst 1997, 122, pp 1347-1352) and Li *et al.* (J. Am. Soc. Mass Spectro. 1997, 8, pp 781-792). The basis of the rejection is reproduced below.

> Aebersold et al. teach a method of identification and quantification of a protein in a sample by cleaving the protein to peptides using a proteolytic enzyme (page 18, pargh. 4) and using a reagent A-L-PRG, wherein A is linked to a solid support (wherein, A comprises biotin, oligohistidine, etc, page 12) and is covalently linked to linker L (L contain metal bound chelate, page 14, 2nd parg. and may contain disulfide group, which is cleavable, page 6, last pargh.); PRG comprises a sulfhydryl group, or an enzyme substrate (page 6, 2nd pargh.) N-hydroxysuccinimide ester groups, etc (claim 32 of Aebersold et al.) to bind to the cleaved peptides. Aebersold et al. teach the use of a tandem technique comprising electrospray ionization mass spectrometry coupled with liquid chromatography (HPLC/ESI-MS/MS (FIG 7), peptide sequence information (page 19, 2nd pargh.) combined with isotope tags for qualitative and quantitative analysis of the protein in a sample. Although Aebersold et al. teach the use of a linker L being labeled with isotopes, they do not label the proteins with said isotope. The A-L-PRG reagent of Aebersold et al (similar to applicants' A-Y-PRG) comprises a chelated metal ion and the stable isotope in their L and use the stable isotope as standard in mass spectrometric analysis. However Aebersold et al. do not use a reagent A-Y-PRG wherein said reagent is not isotopically labeled and hence does not use metal ion as a standard in mass spectrometric analysis.
>
> Use of metal ion as a standard in mass spectrometric studies is well known in the prior art (see page 781, Li et al.). Li et al. teach a well characterized spectra of peptide bound silver ion in mass spectral analysis (Figure 1, page 783.)

It is well known in the art the advantage of purifying and detecting proteins using chelated metal tags comprising various metal ions (Porath et al Prot express and Pur. 1992, 3, 263-281, from IDS) using a variety of chelating agents, such as lanthanide metal ions with DOTA (Moutiez et al). Moutiez et al teach a Gd^{3+} ion chelated to DOTA and teach its separation using metal ion chelate affinity chromatography (page 1350 2nd column) and teach that lanthanide metal complex can be detected using luminescence technique (page 1347 2nd column 2nd paragraph).

Therefore in order to identify and quantify proteins in proteomic samples, one of ordinary skill in the art is **motivated** to modify the A-L-PRG of Aebersold et al with Gd^{3+} DOTA chelate not being modified by isotope label and use the metal ion as standard (as taught by Li et al) in the method of Aebersold et al., because a peptide sample attached to L-PRG with Gd^{3+} DOTA can be separated by metal ion chelate affinity column by HPLC, and optionally can be detected by luminescence before passing into the mass spectrometer.

As such, it would have been obvious to one of ordinary skill in the art to combine the teachings of Aebersold et al., Moutiez et al. and Li et al. to make an A-L-PRG regent having Gd^{3+} DOTA complex in L, use it in the method of identification and quantification of proteins in a sample by a tandem technique comprising electrospray ionization mass spectrometry coupled with liquid chromatography (HPLC/ESI-MS/MS (FIG 7), peptide sequence information using Gd metal ion as standard, and optionally detecting the Gd^{3+} DOTA attached polypeptide by using luminescence before passing the sample into the Mass spectrometer.

1. The rejection fails to establish a *prima facie* case of obviousness for the claimed invention.

As set forth in *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1741, 82 U.S.P.Q.2d 1385, 1396 (2007), a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art. It is also important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements or modify the teachings of the references in order to arrive at the claimed invention. Furthermore, "*there must be some articulated reasoning* with some rational underpinning to support the legal conclusion of obviousness." *KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) (quoting *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006) ("[R]ejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be

some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness")(emphasis added)).

In this case, Appellants respectfully submit that the Patent Office has not established a *prima facie* case of obviousness for the claimed invention. In establishing the rejection, the Patent Office has combined the teachings of Aebersold *et al.* (WO 00/11208), Moutiez *et al.* (1997) and Li *et al.* (1997) and provided nothing more than conclusory statements as to the obviousness of the claimed invention. For example, the rejection of record states:

> Therefore in order to identify and quantify proteins in proteomic samples, one of ordinary skill in the art is **motivated** to modify the A-L-PRG of Aebersold et al with Gd^{3+} DOTA chelate not being modified by isotope label and use the metal ion as standard (as taught by Li et al) in the method of Aebersold et al., because a peptide sample attached to L-PRG with Gd^{3+} DOTA can be separated by metal ion chelate affinity column by HPLC, and optionally can be detected by luminescence before passing into the mass spectrometer.
>
> As such, it would have been obvious to one of ordinary skill in the art to combine the teachings of Aebersold et al., Moutiez et al. and Li et al. to make an A-L-PRG regent having Gd^{3+} DOTA complex in L, use it in the method of identification and quantification of proteins in a sample by a tandem technique comprising electrospray ionization mass spectrometry coupled with liquid chromatography (HPLC/ESI-MS/MS (FIG 7), peptide sequence information using Gd metal ion as standard, and optionally detecting the Gd^{3+} DOTA attached polypeptide by using luminescence before passing the sample into the Mass spectrometer.

Appellants note that there is no articulated reasoning as to why one skilled in the art would have modified the reagents taught in Aebersold *et al.* such that the reagent did not contain an isotope to allow for the identification of a labeled peptide during analysis using mass spectrophotometry. Rather, the Office Actions relied upon conclusory statements that a "peptide sample attached to L-PRG with Gd^{3+} DOTA can be separated by metal ion chelate affinity column by HPLC, and optionally can be detected by luminescence before passing into the mass spectrometer". Appellants submit that the rejections of record fail to articulate any rationale or scientific reasoning for removing isotopic labels from the reagents of Aebersold *et al.* in order to arrive at the presently claimed invention. Furthermore, no explanation is given as to how one is to detect and identify labeled peptides in the absence of the isotopic labels used by Aebersold *et al.* for

that purpose. Accordingly, it is respectfully submitted that a *prima facie* case of obviousness has not been established by the Patent Office and reversal of the rejection is respectfully requested.

2. The cited combination of references fails to teach all the limitations of the claimed invention.

It is fundamental patent law that an obviousness rejection fails if the prior art relied on does not disclose all of the limitations of the claimed invention. *See, e.g., In re Zurko*, 258 F.3d 1379, 1385-86 (Fed. Cir. 2001). Thus, obviousness requires a teaching or suggestion of all limitations in a claim. *CFMT, Inc. v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (citing *In re Royka*, 490 F.2d 981, 985 (C.C.P.A. 1974)).

The claimed invention relates to the utilization of a reagent (A, Y, PRG) that is not isotopically labeled (see currently pending claims 1 and 6). The Office Actions issued to date admit that Aebersold *et al.* fails to teach the claimed reagent (see the Office Action dated August 4, 2009 at page 5 (last sentence), Office Action dated March 4, 2010 at page 4, lines 10-12, Office Action dated July 8, 2010 at page 3, lines 13-15. Moutiez *et al.* (1997) and Li *et al.* (1997) fail to teach reagents meeting this limitation and are directed to other elements of the claims (DOTA-chelated lanthanide metals; Moutiez *et al.*) and Gd^{3+}-labeled DOTA chelates (Li *et al.*). Appellants further note that no reason was identified in the Office Action that would have prompted a person of ordinary skill in the relevant field to modify the teachings of Aebersold *et al.* in the claimed fashion (*i.e.*, to form reagents lacking isotopic labels) and Moutiez *et al.* (1997) and Li *et al.* (1997) do not cure this defect in the rejections of record. Thus, the combined teachings of Aebersold *et al.* (WO 00/11208), Moutiez *et al.* (1997) and Li *et al.* (1997) fail to establish a *prima facie* case of obviousness because each of the limitations of the claims is not taught and no reasoning is given as to why one skilled in the art would have been motivated to modify the reagents of Aebersold *et al.* such that they did not contain the isotopic label necessary for the detection and identification of labeled peptides in the mass spectroscopy step of claims 1 and 6. Accordingly, reversal of the rejection is respectfully requested.

3. The modifications to Aebersold *et al.* proposed in the Office Actions would have rendered the teachings of the reference unsuitable for their intended purpose.

If a proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon*, 733 F.2d 900, 221 U.S.P.Q. 1125 (Fed. Cir. 1984). Appellants respectfully submit that the Examiner has not responded to this line of argument.

The Office Actions have argued that one skilled in the art would have been motivated to alter the teachings of Aebersold *et al.* to use reagents that were not isotopically labeled in combination with metal ions chelated in DOTA in order to identify and quantify proteins (see Office Action dated August 4, 2009 at page 6 (third paragraph), Office Action dated March 4, 2010 at page 5, paragraph 1, Office Action dated July 8, 2010 at page 4, first full paragraph. Appellants respectfully submit that Aebersold *et al.* describe isotope coded affinity tag (ICAT) technology. As discussed in the as-filed specification (see page 4, line 18 through page 5, line 27), ICAT technology relies on (and requires) the use of isotopes in order to allow for the quantification and identification of biological molecules labeled via ICAT technology. ICAT technology is fundamentally different from the present invention (and thus is not similar). Appellants have also argued that modification of the ICAT reagents taught in Aebersold *et al.* would have rendered the reagents of Aebersold *et al.* unsuitable for their intended purpose because the reagents would not have contained the isotopes that allow one to quantify and identify biological molecules in the mass spectroscopy step of claims 1 and 6.

In this case, the modification to the teachings of Aebersold *et al.* proposed in the Office Actions renders the prior art invention unsatisfactory for the analysis of proteins. As noted previously, the present invention differs from the disclosure of Aebersold *et al.* in that the claimed method does not utilize isotopically labeled reagents for the identification of labeled peptides (*i.e.*, the reagent of general formula A-Y-PRG is not isotopically labeled and uses chelate-bound metal ions for differentiation of peptides within a sample). Aebersold *et al.* specifically teach the use of isotopically labeled reagents (see, for example, page 11, first full paragraph and claims 1-19) to identify and/or quantify one or more proteins. Indeed, Aebersold

et al. specifically discuss the comparison of isotopically heavy and light reagents in the practice of their disclosure stating at page 19 (emphasis added):

> In this last step, both the quantity and sequence identity of the proteins from which the tagged peptides originated can be determined by automated multistage MS. This is achieved by the operation of the mass spectrometer in a dual mode in which it alternates in successive scans between measuring the relative quantities of peptides eluting from the capillary column and recording the sequence information of selected peptides. **Peptides are quantified by measuring in the MS mode the relative signal intensities for pairs of peptide ions of identical sequence that are tagged with the isotopically light or heavy forms of the reagent, respectively, and which therefore differ in mass by the mass differential encoded within the affinity tagged reagent.**

Thus, elimination of isotopically labeled reagents would have eliminated one's ability to differentiate between peptides on the basis of mass and rendered the modified methods of Aebersold *et al.* unsuitable for the analysis of proteins. Accordingly, Appellants respectfully submit that there would have been no motivation to modify the teachings of Aebersold *et al.* as proposed in the Office Action since the proposed modification would have rendered the teachings of the reference unsuitable for their intended purpose.

Appellants note the Office Action dated July 8, 2010 argues that it has been admitted that Aebersold *et al.* suggested the use of chelated metal ions. Appellants dispute the characterization of the arguments submitted in the response as an admission. The passage to which the Office Action and the previously filed response refer relates to the use of metal ions for ionization in MS-analysis and each reagent must be labeled with the same metal ion (metal ions having the same mass) in order for the method of Aebersold *et al.* to allow for the identification of peptides on the basis of differing mass (the isotopes in the reagents providing for the ability to differentiate and identify peptides in the mass spectroscopy step). This concept is clearly conveyed by the passage bridging pages 13-14 of Aebersold *et al.* where it is stated that "at least some of the atoms in th elinker groups should be readily replaceable with stable heavy-atom isotopes" and at page 19 (emphasis added):

> In this last step, both the quantity and sequence identity of the proteins from which the tagged peptides originated can be determined by automated multistage MS. This is achieved by the operation of the mass spectrometer in a dual mode in which it alternates in successive scans between measuring the

relative quantities of peptides eluting from the capillary column and recording the sequence information of selected peptides. **Peptides are quantified by measuring in the MS mode the relative signal intensities for pairs of peptide ions of identical sequence that are tagged with the isotopically light or heavy forms of the reagent, respectively, and which therefore differ in mass by the mass differential encoded within the affinity tagged reagent.**

4. The obviousness rejection of record is the result of improper hindsight reconstruction of the claimed invention.

A fact finder should be aware of the distortion caused by hindsight bias. *KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727, 1742 (2007) and "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *KSR,* 127 S.Ct at 1741. Furthermore, "*there must be some articulated reasoning* with some rational underpinning to support the legal conclusion of obviousness." *KSR Int'l v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) (quoting *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006) (emphasis added)). Appellants respectfully submit that the Examiner has not responded to this line of argument.

As discussed above and admitted in the Office Actions, the use of reagents containing no isotopes (even if chelated metals are present) is neither disclosed nor proposed by Aebersold *et al.* In fact, the actual analysis taught by Aebersold *et al.* is based on the use of isotopes "integrated" into the reagents used in the methods taught by the reference. Thus, in order to arrive at the present invention, starting from Aebersold *et al.*, the person of skill had to:

1) disregard the main technical feature of Aebersold *et al.*, namely the tagging of biological molecules using differentially isotopically labeled reagents (without a chelator being involved) and create reagents that were not isotopically labeled,
2) add and permanently include a chelator into the L-group of Aebersold *et al.*, and
3) include a lanthanide metal ion as the label (see Response filed June 4, 2010 at pages 8-9).

In the absence of the claimed invention, there is no motivation in Aebersold *et al.* to modify the A-L-PRG-affinity tagged protein reactive reagents in *any* of the three ways (much less in all three of the ways) described above and claimed in this matter and no rationale

explaining why one skilled in the art would have been motivated to proceed in such a fashion has been articulated in any of the Office Actions. Furthermore, none of the additional references teach or suggest the modification of Aebersold *et al.* in such a fashion (their teachings relating to the use of DOTA-chelated lanthanide metals (Moutiez *et al.*) and Gd^{3+}-labeled DOTA chelates (Li *et al.*)). Indeed, modification of Aebersold *et al.* in order to arrive at the claimed invention as proposed in the Office Actions would have rendered the teachings of the Aebersold *et al.* reference unsuitable for its intended purpose and no articulated reasoning has been provided as to why one of ordinary skill in the art would have proceeded in such a fashion. Thus, Appellants respectfully submit that the Office Actions have engaged in improper hindsight reconstruction of the claimed invention and reversal of the rejection is respectfully requested.

B. Conclusion

In view of the foregoing, the Appellants urge the Board to reverse the outstanding rejection under 35 U.S.C. § 103(a) and pass this application to issuance.

Respectfully submitted,

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VIII. CLAIMS APPENDIX

Claims 1 and 6 were amended by Amendment dated June 4, 2010.

Claims 1, 2, 6, 25, 31 were amended and claims 39 and 40 were added by Amendment dated November 20, 2009.

Claims 1 and 6 were amended, claims 7 and 23 were canceled and claims 35-38 were added by Amendment dated April 3, 2009.

Claims 1, 2 and 6 were amended, claims 9-21 were canceled and claims 22-34 were added by Amendment dated September 2, 2008.

Claims 1 and 6 were amended by Amendment dated January 8, 2008.

Claim 2 was amended by Amendment dated June 18, 2007.

Claims 1-21 were amended by Amendment dated December 20, 2004.

1 (previously presented). A method for the identification and/or quantification of one or more proteins derived from the proteome of a cell in a sample containing a mixture of such proteins, wherein said method comprises the steps of:

a) providing a sample which contains a mixture of proteins;

b) providing a reagent for the analysis of peptides which comprises A, Y and PRG in which

A constitutes at least one functional group for the reversible, covalent or non-covalent binding to a support material,

Y is a group comprising at least one chelate function for metals, and comprising a metal ion bound thereto wherein the metal is selected from the group consisting of Ag, Al, As, Au, Be, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Hg, Ho, In, La, Li, Lu, Mn, Na, Nd, Ni, Pb, Pr, Rb, Rd, Sb, Sm, Sn, Tb, Tl, Tm, V, W, Y, Yb and Zn;

PRG is a reactive group for the selective binding to peptides or other biomolecules to be analyzed; and wherein the arrangement of A, Y, and PRG is interchangeable and said reagent is not isotopically labeled;

c) chemically or proteolytically cleaving the proteins in the sample in order to produce peptides;

d) coupling the peptides to the reagent of step b) wherein the peptides are labeled by the reagent;

e) selecting the peptides labeled in step d) using a functional group for the reversible, covalent or non-covalent binding to a support material and removal of unbound peptides;

f) releasing the bound peptides from the support material and elution from the matrix; and

g) detecting and identifying the labeled peptides by means of mass spectrometry.

2 (previously presented). The method, according to claim 1, wherein the cleavage of the proteins is performed enzymatically by a proteolytic enzyme.

3 (previously presented). The method, according to claim 1, wherein the labeled peptides, after their release from the support material and before their analysis by mass spectrometry, are separated from each other by means of HPLC.

4 (previously presented). The method, according to claim 1, characterized in that several protein- and/or peptide-containing samples are analyzed together.

5 (previously presented). The method, according to claim 1, further comprising the sequencing of the labeled peptides.

6 (previously presented). A method for the detection of the relative expression of proteins in a protein-containing sample, wherein said method comprises the steps of:

a) providing a biological sample which contains proteins;

b) providing a reagent for the analysis of peptides which comprises A, Y and PRG in which

A constitutes at least one functional group for the reversible, covalent or non-covalent binding to a support material,

Y is a group comprising at least one chelate function for metals, and comprising a metal ion bound thereto wherein the metal is selected from the group consisting of Ag, Al, As, Au, Be, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Hg, Ho, In, La, Li, Lu, Mn, Na, Nd, Ni, Pb, Pr, Rb, Rd, Sb, Sm, Sn, Tb, Tl, Tm, V, W, Y, Yb and Zn,

PRG is a reactive group for the selective binding to peptides or other biomolecules to be analyzed and said reagent contains no isotopes; and wherein the arrangement of A, Y, and PRG is interchangeable and said reagent is not isotopically labeled;

c) chemically or proteolytically cleaving the proteins in the sample in order to produce peptides;

d) coupling the peptides to the reagent of step b) wherein the peptides are labeled by the reagent;

e) selecting the peptides labeled in step d) utilizing a functional group for the reversible, covalent or non-covalent binding to a support material and removal of the unbound peptides;

f) releasing the bound peptides from the support material and elution from the matrix;

g) detecting and identifying the labeled peptides by means of mass spectrometry; and

h) measuring the relative occurrence of the differently labeled peptides as distinct peaks of ions in order to determine the relative expression of the protein, from which the labeled peptide is derived.

7 (canceled).

8 (previously presented). The method, according to claim 6, characterized in that the labeled peptides are detected by means of a tandem technique selected from the group consisting

of matrix-assisted laser desorption/ionization (MALDI), time-of-flight (TOF)-TOF-MS and electrospray ionization (ESI)-MS.

9-21 (canceled).

22 (previously presented). The method according to claim 1, wherein said mixture of proteins has not been labeled with an isotope.

23 (canceled).

24 (previously presented). The method according to claim 1, wherein the PRG group is selected from the group consisting of sulfhydryl-reactive groups, amine-reactive groups and enzyme substrates.

25 (previously presented). The method according to claim 1, wherein the PRG group is selected from the group consisting of amine-reactive pentafluorophenyl ester groups, amine-reactive N-hydroxysuccinimide ester groups, sulfonylhalides, isocyanates, isothiocyanates, active esters, tetrafluorophenyl esters, acid halides, acid anhydrides, homoserine lactone-reactive primary amine groups, carboxylic acid-reactive amines, alcohols, 2,3,5,6-tetrafluorophenyltrifluoro-acetates, iodine acetylamide groups, epoxides, α-haloacyl groups, nitriles, sulfonated alkyls, arylthiols and maleimides.

26 (previously presented). The method according to claim 1, wherein A is selected from the group consisting of biotin, modified biotin, 1,2-diols, glutathiones, maltoses, nitrilotriacetic acid groups, oligohistidines and haptens.

27 (previously presented). The method according to claim 1, further comprising a linker between the groups A, Y and/or PRG that is cleavable.

28 (previously presented). The method according to claim 27, wherein the linker contains a disulfide group.

29 (previously presented). The method according to claim 6, wherein said mixture of proteins has not been labeled with an isotope.

30 (previously presented). The method according to claim 6, wherein the PRG group is selected from the group consisting of sulfhydryl-reactive groups, amine-reactive groups and enzyme substrates.

31 (previously presented). The method according to claim 6, wherein the PRG group is selected from the group consisting of amine-reactive pentafluorophenyl ester groups, amine-reactive N-hydroxysuccinimide ester groups, sulfonylhalides, isocyanates, isothiocyanates, active esters, tetrafluorophenyl esters, acid halides, acid anhydrides, homoserine lactone-reactive primary amine groups, carboxylic acid-reactive amines, alcohols, 2,3,5,6-tetrafluorophenyltrifluoro-acetates, iodine acetylamide groups, epoxides, α-haloacyl groups, nitriles, sulfonated alkyls, arylthiols and malcimides.

32 (previously presented). The method according to claim 6, wherein A is selected from the group consisting of biotin, modified biotin, 1,2-diols, glutathiones, maltoses, nitrilotriacetic acid groups, oligohistidines and haptens.

33 (previously presented). The method according to claim 6, further comprising a linker between the groups A, Y and/or PRG that is cleavable.

34 (previously presented). The method according to claim 33, wherein the linker contains a disulfide group.

35 (previously presented). The method according to claim 1, wherein Y is selected from the group consisting of macrocyclic lanthanoid chelate complexes, functionalized tetraaza-macrocycles, polyaza-polyacetic acids, DOTA, DOTA-derivatives, NOTA, NOTA-derivatives, 1,4,7,10,13,16,19,22-octaazacyclotetracosane-1,4,7,10,13,16,19,22-octaacetic acid (OTEC),

1,4,7,10,14-17,20,23-octaazacyclohexacosane-1,4,7,10,14,17,20,23-octaacetic acid (OHEC), EDTA, DTPA-BP, DTPA, DO3A, HP-DO3A and DTPA-BMA.

36 (previously presented). The method according to claim 6, wherein Y is selected from the group consisting of macrocyclic lanthanoid chelate complexes, functionalized tetraaza-macrocycles, polyaza-polyacetic acids, DOTA, DOTA-derivatives, NOTA, NOTA-derivatives, 1,4,7,10,13,16,19,22-octaazacyclotetracosane-1,4,7,10,13,16,19,22-octaacetic acid (OTEC), 1,4,7,10,14-17,20,23-octaazacyclohexacosane-1,4,7,10,14,17,20,23-octaacetic acid (OHEC), EDTA, DTPA-BP, DTPA, DO3A, HP-DO3A and DTPA-BMA.

37 (previously presented). The method according to claim 35, wherein Y is a macrocyclic lanthanoid chelate complex.

38 (previously presented). The method according to claim 36, wherein Y is a macrocyclic lanthanoid chelate complex.

39 (previously presented). The method according to claim 1, wherein said metal is selected from the group consisting of Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu.

40 (previously presented). The method according to claim 6, wherein said metal is selected from the group consisting of Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu.

IX. EVIDENCE APPENDIX

None.

X. RELATED PROCEEDINGS APPENDIX

None.